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THE EFFECTS OF EDTA AND EGTA ON RENIN SECRETION

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1 The effects of the disodium salt of ethylenediamine tetra-acetate (EDTA) and 1,2,bis,2 amino-ethoxyethane-*NNN'N'*-tetra-acetic acid (EGTA) on renin secretion and vascular resistance were studied in the isolated perfused kidney of the rat.

2 Both substances produced a significant increase of renin release.

3 In the absence of calcium and magnesium, EDTA still increased renin release and there was now a considerable increase of perfusion pressure.

4 The rise of pressure but not the rise of renin was inhibited by the removal of potassium from the perfusate when EDTA was administered in the absence of calcium.

5 Propranolol and phenoxybenzamine had no effect on the vasoconstrictor action of EDTA.

6 EGTA was less effective as a renin releaser than EDTA until magnesium was removed from the perfusate. Further, it had only a small effect on perfusion pressure in contrast to EDTA.

Introduction

The probable importance of calcium ion in the release of renin from the juxtaglomerular cells has been stressed in recent work from this laboratory. It was shown that lowering the external calcium concentration increased renin release and the inhibition of renin release produced by angiotensin was calcium-dependent (Vandongen & Peart, 1974a), and that lanthanum, which is known to block calcium flux across various cell membranes (Weiss, 1974), inhibits spontaneous renin release as well as that induced by isoprenaline and glucagon (Logan, Tenyi, Quesada, Peart, Breathnach & Martin, 1975). This latter inhibition was only overcome by the introduction of the disodium salt of ethylenediamine tetra-acetate (EDTA) and it was suggested that this might act by chelating and therefore removing lanthanum on the surface of the juxtaglomerular cells and then increasing calcium efflux. It therefore seemed important to study the actions of EDTA and 1,2,bis, 2-aminoethoxyethane-*NNN'N'*-tetra-acetic acid (EGTA), which have different affinities for calcium (Williams, 1972), on renin release, and at the same time to note their actions on renal vasoconstriction as a possible indicator of calcium flux since vasoconstriction is probably associated with the entry or increase of calcium within smooth muscle cells (Somlyo & Somlyo, 1968; Keatinge, 1972; Van Breemen, Farinas, Casteels, Gerba, Wuytack & Deth, 1973). The likely origin of juxtaglomerular cells from vascular smooth muscle cells (Barajas & Latta, 1967) makes this comparison especially apposite.

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Methods

Kidney perfusion

Male Wistar rats (300–350 g) maintained on a normal diet, were anaesthetized with sodium pentobarbitone (0.1 mg/g) intraperitoneally and given 100 units heparin intravenously. The left kidney was isolated and perfused as described previously (Vandongen, Peart & Boyd, 1973). The perfusion fluid was usually 'Krebs-Ringer dextran' of the following composition (mmol/l): Na 135, Ca 3.7, K 6.0, Mg 1.2, glucose 10 and dextran 36 g/l (mol wt 70,000). This was equilibrated with 95% O_2 and 5% CO_2 at 37°C, and was delivered as pulsatile flow at a constant rate (usually 8 ml/min) by roller pump. Perfusion pressure was measured by transducer and Devices M2 recorder. The perfusion fluid was modified for different experiments and calcium and magnesium were omitted without ionic replacement. When potassium was omitted the composition of the fluid was (mmol/l): Na 135, Ca 2.5, choline chloride 5.5, Mg 1.0, glucose 10 and dextran 36 g/l (mol wt

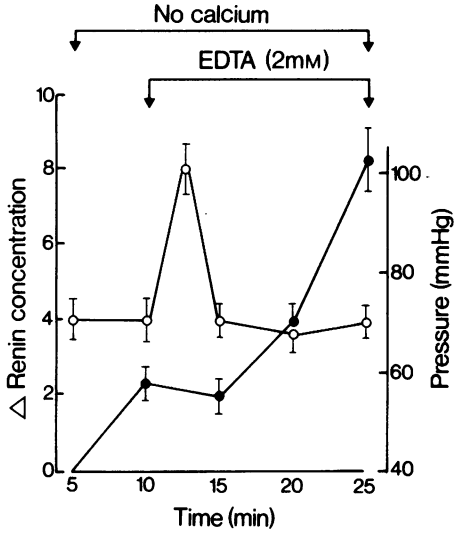

Figure 1 The effect of EDTA in the absence of calcium on renin concentration (●) and renal perfusion pressure (○) in the isolated kidney of the rat.

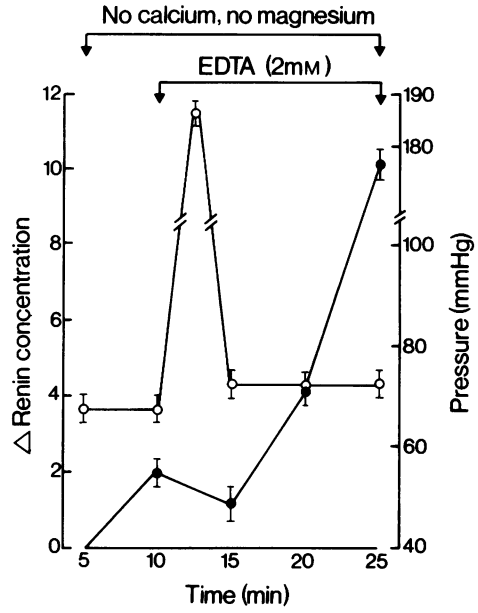

Figure 2 The effect of EDTA in the absence of calcium and magnesium on renin concentration (●) and renal perfusion pressure (○) in the isolated kidney of the rat. Note the break in the scale for pressure due to the large rise.

70,000), buffered with Hepes (N-2-hydroxyethyl-piperazine-*N'*-2-ethane-sulphonic acid) (Sigma) to pH 7.4 and oxygenated with 100% O_2, and the control solution had KCl (K 6.0 mmol/l) in place of the choline chloride. Ethylenediamine tetra-acetate disodium salt (EDTA) and 1,2,bis,2 aminoethoxy-ethane-*NNN'N'*-tetra-acetic acid (EGTA) (Hopkin and Williams) were added to the perfusion fluids in various experiments in concentrations of 1 or 2 mmol/litre. The osmolarity of the buffers was checked by a freezing point depression method (Advanced Osmometer 3L; Advanced Instruments Inc., U.S.A.). Phenoxybenzamine (Smith Kline and French) and propranolol (ICI) dissolved in the appropriate perfusion fluid were infused separately into the arterial line through a needle in the tubing at 0.04 ml/minute.

Renin assay

One ml from each perfusate sample (8 ml) was dialysed successively at pH 5 and pH 7.5 for 24 h at 4°C against phosphate buffers containing gentamicin (10 μg/ml) and EDTA to remove angiotensinases (Skinner, 1967). Samples were then incubated at 37°C with plasma from rats nephrectomized 24 h previously (1 ml perfusate with 0.4 ml plasma) which had been treated by similar dialysis procedures. The reaction was stopped by heating to 90°C for 10 minutes. The angiotensin I produced was measured by radio-immunoassay (Boyd, Adamson, Fitz & Peart, 1969), and the renin activity expressed as nmol of 5-isoleucine-angiotensin I generated l^{-1} h^{-1}. The results were expressed as the ratio of the renin concentration at observed time to the renin concentration at zero time (△). All values given are means ± s.e. and significance was measured by Student's paired *t* test.

Types of infusion

Initial experiments were carried out in which control infusions were made with the following solutions: Krebs ($n=16$); calcium-free ($n=5$); magnesium-free ($n=5$); calcium and magnesium-free ($n=5$); Hepes ($n=5$). In successive experiments, after a control period of 10 min the perfusion fluid was changed to one containing either EDTA or EGTA and the effect on perfusion pressure and renin concentration measured during the next 15 minutes. In two other sets of experiments, the Krebs solution contained either propranolol (4 nmol/ml) ($n=5$) or phenoxy-benzamine (0.5 nmol/ml) ($n=6$) throughout the control period and was continued when the EDTA was introduced.

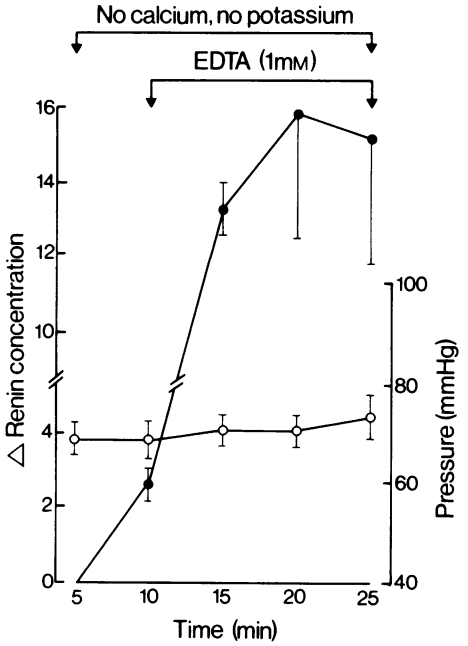

Figure 3 The effect of EDTA in the absence of calcium and potassium on renin concentration (●) and renal perfusion pressure (○) in the isolated kidney of the rat.

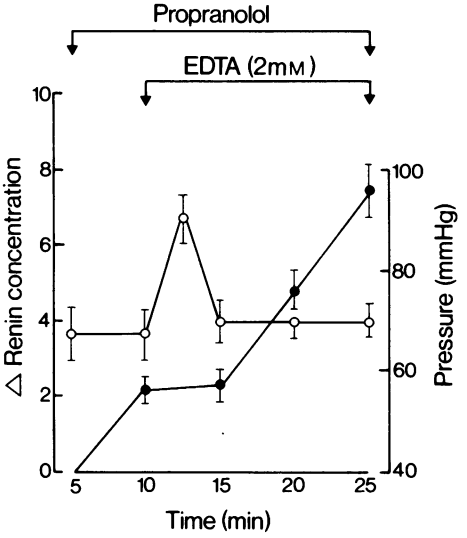

Figure 4 The effect of propranolol (4 nmol/ml) on renin concentration (●) and renal perfusion pressure (○) in the isolated kidney of the rat.

Results

Control infusions

Comparison was made between the renin concentration in the sample collected after 5 min perfusion and after 10, 15, 20 and 25 min, and since the maximum difference was seen at 25 min, the result was expressed as the ratio of the concentrations at that time over the 5 min value. Krebs solution ($n=16$; △ 2.1 ± 0.2); calcium-free ($n=5$; △ 2.7 ± 0.34); magnesium-free ($n=5$; △ 2.2 ± 0.1); calcium and magnesium-free ($n=5$; △ 1.24 ± 0.48); Hepes solution ($n=5$; △ 1.67 ± 0.4). Since the changes observed with EDTA and EGTA were very much greater, comparisons thereafter were not made with these controls but the differences within each experiment from the starting value were used. There were no changes of pressure with time in any of this group of experiments.

EDTA with different solutions

Calcium free ($n=8$) (Figure 1). The addition of EDTA (2 mmol/l) caused an immediate rise of pressure without a change in renin release followed by a gradual rise in renin over the next 10 min ($P<0.001$), while the pressure returned to its initial value within 5 minutes.

Calcium and magnesium-free ($n=5$) (Figure 2). The addition of EDTA (2 mmol/l) was associated with a very marked rise of pressure and a significant reduction in renin release ($P<0.05$). Continued perfusion led to a drop in pressure almost to the starting value within 5 min with a steady rise in renin secretion over 10 min ($P<0.001$).

Calcium and potassium-free ($n=4$) (Figure 3). The addition of EDTA did not cause a change of pressure in contrast to experiments where potassium was present (Figure 1) and renin release occurred promptly (within 5 min) (contrast Figure 1), and continued to rise over 10 min ($P<0.001$).

Krebs solution with propranolol ($n=5$) *or phenoxybenzamine* ($n=6$) (Figures 4 and 5). When EDTA (2 mmol/l) was introduced in the presence of either propranolol (4 nmol/ml) or phenoxybenzamine (0.5 nmol/ml), the immediate short-lived rise in pressure was unaffected and renin concentration rose significantly after the first 5 min as in the experiments in the absence of these drugs ($P<0.001$) (c.f. Figure 1).

EGTA with different solutions

Calcium-free ($n=5$) (Figure 6). EGTA (2 mmol/l) caused an immediate rise in renin secretion which was

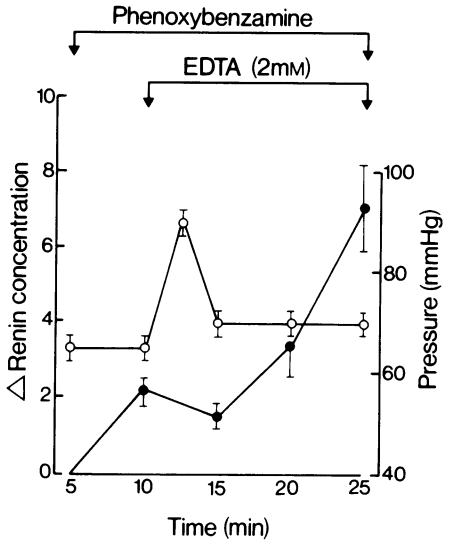

Figure 5 The effect of phenoxybenzamine (0.5 nmol/ml) on renin concentration (●) and renal perfusion pressure (○) in the isolated kidney of the rat.

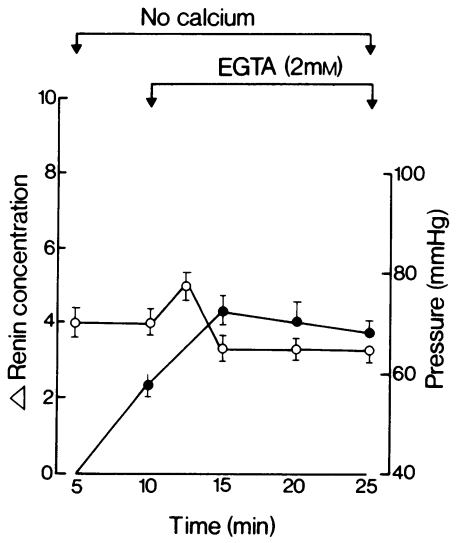

Figure 6 The effect of EGTA in the absence of calcium on renin concentration (●) and renal perfusion pressure (○) in the isolated kidney of the rat.

not progressive after the first 5 min but remained significantly elevated ($P < 0.01$). The immediate rise of pressure was very slight ($P > 0.05$) and returned to a maintained level within 5 minutes.

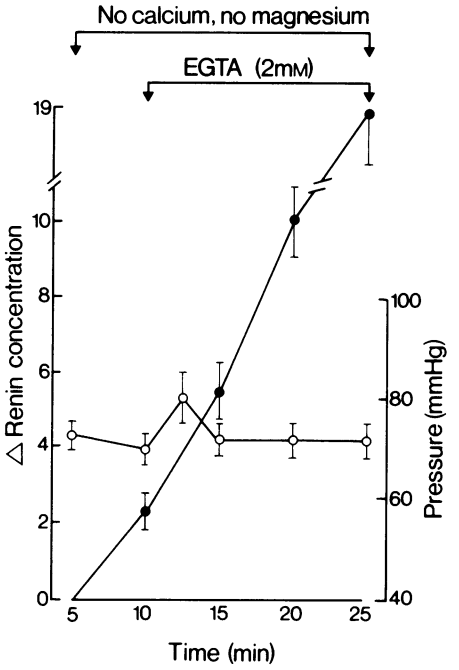

Figure 7 The effect of EGTA in the absence of calcium and magnesium on renin concentration (●) and renal perfusion pressure (○) in the isolated kidney of the rat. Note the break in the renin concentration line because of the marked increase.

Calcium and magnesium-free ($n = 4$) (Figure 7). In contrast to the previous experiments, the introduction of EGTA (2 mmol/l) caused a rapid and increasing rise of renin secretion ($P < 0.001$). The pressure change was insignificant and transient ($P > 0.05$) and stayed on the baseline after 5 minutes.

Discussion

In previous studies on the perfused kidney of the rat there was evidence that EDTA increased renin release (Vandongen & Peart, 1974a; Logan *et al.*, 1975; Logan, Tenyi, Peart, Breathnach & Martin, 1976). In the present series of experiments it is clear that EDTA in the absence of external calcium and magnesium caused a rise of renin release which was continued to the termination of the experiments. There was no significant difference between the experiments without calcium and without calcium and magnesium (Figures 1 and 2). The addition of propranolol or phenoxybenzamine when the perfusate contained both calcium and magnesium, did not prevent this steady rise in renin release due to EDTA (Figures 4 and 5). This

therefore excludes the participation of α- or β-adrenoceptor stimulation in this renin release. The effects of EDTA on perfusion pressure in this series of experiments is of great interest since the transient rise of pressure produced even in the absence of calcium (Figure 1) was greatly magnified in the absence of both calcium and magnesium (Figure 2) and this latter rise, which was confined to the first 5 min of EDTA perfusion, was associated with a significant fall in renin release. Can these findings be related to the hypothesis that renin inhibition is associated with an increased influx or rise of intracellular calcium ion (Vandongen & Peart, 1974b; Logan *et al.*, 1975; 1976)? It is conceivable that if EDTA caused a marked calcium gradient from the interior to the exterior of the cell, intracellular calcium stores would be released, leading to smooth muscle contraction, and in the juxtaglomerular cell to inhibition of renin release. As efflux increased there would be a subsequent fall of intracellular calcium, smooth muscle would relax and renin release would be increased. Certainly the contraction was not blocked by phenoxybenzamine and is presumably not mediated through α-receptors. The effect of omitting potassium as well as calcium from the perfusate in the presence of EDTA (Figure 3) is of considerable interest since this abolished the rise of pressure, and the renin release was quicker and of the same eventual magnitude as in previous experiments (compare Figures 1 and 3). One possible explanation could be that external potassium is controlling calcium influx or release from the cell membrane and that in its absence efflux is favoured and renin release is therefore quicker (Figure 3). The cell membrane or intracellular processes within the smooth muscle and juxtaglomerular cell must be involved in the actions of EDTA and EGTA since purely removing calcium and magnesium from the perfusing medium with normal potassium content had a much smaller effect on renin release and none on pressure.

The effect of EGTA, which has a much greater affinity for calcium than for magnesium (Williams, 1972), therefore becomes of great importance and while it certainly stimulated renin release in the absence of calcium, it was not as active as EDTA (Figures 6 and 7) and the difference between renin levels at 25 min in the two series of experiments was significant ($P < 0.005$). The omission of magnesium and calcium from the perfusate in the presence of EGTA then led to an immediate and continuous rise in renin release. The small pressor effect with EGTA in the absence of calcium had an inhibitory action on renin release. The small pressor effect with EGTA further indicates that there is unlikely to be any big intracellular shift of calcium ion in contrast to the results with EDTA (compare Figures 1 and 6). It is possible that an antagonism between external calcium and magnesium in relation to smooth muscle contraction is also revealed by the different effects of EDTA and EGTA (compare Figures 1, 2, 6 and 7). It is realized that interpretations of a complicated ionic interrelationship of this sort are dangerous and by concentrating on the possible importance of the flux and intracellular concentration of calcium ion, that other effects might be given too little emphasis. A rise of renin release might simply be due to increased cell membrane permeability caused by EDTA or EGTA without even the necessity for changes in ionic flux. Equally EDTA or EGTA may be active inside the cell and apart from manipulation of external ionic concentrations, there is no direct evidence presented here. The importance of magnesium in smooth muscle contraction has recently received more attention (Ford & Podolsky, 1970) and in other arteries of the rat, a relation between magnesium efflux and a ouabain-sensitive sodium pump has been postulated (Palatý, 1974). The vasoconstrictor effects of angiotensin which are certainly abolished in the rat kidney preparation by removal of calcium from the perfusate (Vandongen & Peart, 1974a), are increased by omitting magnesium in other preparations (Altura & Altura, 1971). Together with the present evidence, a relation between calcium, magnesium and potassium in respect of both smooth muscle contraction and renin release is strongly indicated by these postulated actions of EDTA and EGTA.

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